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Frontal fibrosing alopecia: reflections and hypotheses on etiology and pathogenesis

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Abstract: Since it was first described by Kossard in 1994, frontal fibrosing alopecia (FFA) has been something of an enigma. The clinical heterogeneity of FFA, its apparent rarity, and investigators' suboptimal access to phenotypically consistent patient cohorts, may all have had a negative impact on delineating disease pathogenesis. Moreover, there is a relative paucity of epidemiologic interventional and basic research studies, and there have been no advances in translational therapeutics, unlike for other inflammatory dermatoses, such as alopecia areata (AA). Dermatologists anecdotally describe an increasing incidence in FFA over the last decade, which has led to the notion that the disorder may be induced by unknown environmental triggers. On the other hand, segregation of FFA in some families lends support to an unexplored genetic element implicated in disease pathogenesis. We herein review what is known about the pathobiology of FFA and formulate working hypotheses to advance insight into this intriguing hair disorder.

Keywords: FFA; frontal fibrosing alopecia; LPP; lichen planopilaris; scarring; cicatricial alopecia

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Introduction

Cicatricial or scarring alopecias, whether primary or secondary, are a therapeutically challenging problem in clinical practice. Primary cicatricial alopecias (PCA) represent a rather diverse group of inflammatory disorders, where the common denominator, in pathologic terms, is the irreversible destruction of hair follicles. Frontal Fibrosing Alopecia (FFA) is a recently described, predominantly postmenopausal, primary lymphocytic cicatricial alopecia (1) that has been thought to be a variant of lichen planopilaris (LPP) (2). Clinically, the disorder involves the frontotemporal hairline with perifollicular erythema, follicular hyperkeratosis and scarring with evident hairline recession, although clinical signs of inflammation are not always evident (3). Eyebrow loss is recorded in 50%-83% of cases (3–5) but body hair loss has been less commonly reported, as has eyelash volume loss (0%-77%) (5-7). The histology of FFA (**Fig 1**) is similar to that of classic (multifocal) LPP, with the early active stages showing a perifollicular lichenoid inflammatory infiltrate involving the isthmus and infundibulum; later stages are characterized by perifollicular fibrosis with follicular dropout and follicular scars replacing the pilosebaceous units (2, 8). The inflammatory focal point in FFA is the bulge area of the hair follicle (HF) in contrast to alopecia areata (AA),

where the bulb at the lower third of the HF is at the epicentre (9, 10). The identical histological appearance accounts for the widespread acceptance of FFA as clinical sub-variant of (follicular) lichen planus (11).

Dividing PCAs histologically into lymphocytic, neutrophilic and mixed, has guided clinical classification but microscopic characterisation may not reflect upstream differences in pathogenesis. Neither do the histological similarities reflect clinical overlap: the classic pattern of LPP behaves differently clinically and epidemiologically from its rather distinct subvariant FFA: the latter has a striking clinical pattern of ear-to-ear scarring hair loss, often with eyebrow and body hair involvement and much commoner in postmenopausal women (12, 13). Only rarely does FFA affect men whereas LPP does not show a gender or older age predilection. Of note, pediatric cases of LPP have also been reported, whereas FFA is unheard of in the pediatric population (14).

The exact pathogenesis of FFA remains unknown. Its postmenopausal occurrence led to the speculation of a hormone-related triggering mechanism. Moreover, 5-alpha reductase inhibitors have been reportedly used with some success, although it has been argued that the beneficial effect of these may be on any concomitant androgenetic element of hair loss, while the overall evidence-basis for this claim remains poor (12, 15). There have been familial cases of FFA reported in the literature (16–19) but robust genetic analysis of the condition has not been undertaken to date.

The case for a genetic basis underlying FFA and the role of the environment

Genetic exploration has been instrumental in revealing molecular pathways underlying alopecic variants, such as Marie-Unna hypotrichosis (20) and alopecia areata (AA) (21), among other cutaneous disorders. It would therefore be prudent to consider the research question of whether there is genetic basis to be explored and, if so, what method would be more appropriate to employ in doing so.

The notion that FFA is underlain by a genetic element is supported primarily by evidence of disease segregation in first-degree relatives. We and others (16–19) have reported familial cases of FFA, where the observed inheritance is in keeping with an autosomal dominant

mode with incomplete penetrance, lending support to the hypothesis that the trait in question may be Mendelian. The exceptionally rare incidence of the disorder would also be in keeping with the hypothesis that FFA is caused by rare alleles of large effect size. Twin studies can be insightful in discerning whether a trait is strongly genetic and we have undertaken a search in the UK via our network of collaborating clinicians and Twins UK: 3 pairs of monozygotic (MZ) twins affected by FFA have been identified, 2 of which are concordant and 1 discordant (67% concordance vs 33% discordance) in a cohort of just over 12,000 individuals. It should be emphasized that the rare overall incidence of FFA and the small number of twin pairs identified (N=3) warrant caution in interpreting the above observation. Moreover, even strongly genetic conditions, such as systemic lupus erythematosus (SLE), are characterized by higher discordance among MZ twins (22), thereby supporting the suggestion that epigenetics may play a profound role in FFA pathogenesis in addition to genetic predisposition. Nevertheless, case-studying of MZ cases could be insightful when undertaken with robust research methods.

On the other hand, the apparent recent increase in the overall incidence of FFA has sparked interest in 'environmental' triggers (23). The late onset of FFA could imply lower genetic causality, although there are plenty of disorders, which are characterized by late life onset and yet which are known to be genetically predisposed, such as systemic lupus erythematosus (SLE) (24) and hidradenitis suppurativa (HS) (25). It is worth noting that, despite the later-life manifestations of the classic frontal fibrosing pattern of hair loss, individuals affected by FFA report, in our experience, lifelong body hair loss, predating eyebrow and scalp involvement. This diffuse and perhaps unrecognized long-term hair loss would also be in keeping with germline predisposition, enhanced by a more complex interplay of possible hormonal factors postmenopause.

Parallels between FFA and AA

The concept of immune-privileged anatomical sites has been well-established since Medawar's experiments, which demonstrated that allogeneic skin grafts are not subjected to immunological rejection when transplanted into certain sites, such as the eye or brain (26). Within the skin, the hair follicle (HF) is a site of relative immune privilege (IP), which serves as a protective strategy against autoimmune injury. Protection is achieved by

downregulation of major histocompatibility complex (MHC) class I and β2 microglobulin in the bulb and bulge of the hair follicle epithelium, besides an augmented expression of a local immunosuppressive milieu. In physiological terms, the exact role of the HF IP remains somewhat unclear, although proposed theories have included the possibility of the crucial evolutionary benefit of ensuring mammals such as polar bears maintained their hair (27–33). Relative IP loss in anagen hair follicular epithelium is postulated to underlie the organ-specific auto-immune disorder AA, where loss of relative IP leads to ectopic MHC class I expression and, as yet elusive, autoantigens trigger a CD8+T cell-mediated inflammatory process, microscopically seen as the classic peribulbar 'swarm of bees' (**Fig 2**) (34–36).

The IP-privileged HF bulge is home to epithelial hair follicle stem cells (37). The crucial role of epithelial hair follicle stem cells (eHFSC) in the pathobiology of PCA is well established: destruction of the bulge is detrimental to the HF's regenerative ability and is seen in permanent, scarring types of alopecia (38, 39). Harries *et al.* studied how proinflammatory events influence eHFSC integrity and postulated that LPP (and variant FFA) represents a T-cell dependent autoimmune disorder, underlain by IFNγ–Induced IP collapse at the level of the bulge (40). IP collapse was evidenced by reduced expression of the IP guardians $TGF\beta_2$ and CD200 and enhanced expression of the normally locally down-regulated presence of MHC class I/II and β_2 microglobulin, which are thought to limit autoantigen presentation (41). In other words, LPP and variant FFA were postulated to mirror the autoimmune process underlying AA with the difference that IP in AA is centred at the deeper but less catastrophic potential bulb, as opposed to the critical stem cell niche and anatomically more superficial bulge. The observed features were only found to characterise lesional areas of scalp skin in individuals affected by LPP, whereas the previously proposed crucial role of defective PPARγ signalling, which Karnik and colleagues (42) found to underlie both lesional and non-lesional scalp skin, could not satisfactorily explain the pattern of patchy involvement in LPP. On the other hand, as Harries *et al.* pointed out, that study did not allow one to conclude whether IFNγ production preceded CD8+ T cell infiltration at the bulge level or whether elusive factors attract T cells, which would subsequently secrete IFNγ, thereby causing IP collapse (41).

Genetic determinants in the pathogenesis of AA were revealed with the help of the genome-wide association study led by Christiano and colleagues: a region of strong association within the novel ULBP gene cluster on chromosome 6q25.1 was identified, which encodes activating ligands of the natural killer cell receptor NKG2D (21). When probed, it was demonstrated that ULBP3 was expressed in lesional scalp and upregulated in the HF dermal sheath during active disease stages (21). If we consider LPP and variant FFA to be immunologically analogous to a bulge-affecting AA-like process, it might be anticipated that the causative weak link in LPP and variant FFA could be reminiscent to *ULBP3*: an overexpressed 'danger signal' or a defective 'gate-keeper' at the level of the bulge, responsive to stressors that might impose a breach of the integrity of the follicular unit. Such a locus could be explored using an investigational genomic approach.

Can we get clues about FFA from other fibrotic disorders?

Fibrotic disorders comprise a multitude of clinical entities, ranging from widespread systemic sclerosis (43, 44), multifocal fibrosclerosis (45, 46), sclerodermatous graft-versus-host disease (47) and nephrogenic systemic fibrosis (48, 49) to more organ-specific disorders such as cutaneous, pulmonary, hepatic, and renal fibrosis (7, 50–52). Whether reactive or reparative, these fibrotic disorders are defined by excessive fibrous connective tissue and aberrant and excessive deposition of extracellular matrix (ECM) in involved tissues. Enhanced expression of genes encoding matrix proteins is a common finding in many of these conditions, where the resulting fibrosis disrupts normal architecture, ultimately leading to organ dysfunction and failure (53–55). A common feature is a dense population of activated fibroblasts or myofibroblasts, which are largely responsible for the hyper-fibrotic response by promoting the production of fibrillar type I and type III collagens and reducing expression of genes encoding extracellular matrix (ECM)–degradative enzymes (56–59). Although the exact origins of the mesenchymal cells leading the aberrant collagen and ECM protein production has not been entirely elucidated, resident fibroblasts, bone-marrow derived fibrocytes, as well as endothelial and epithelial cells undergoing epithelial-to-mesenchymal transition (EMT) are important players in the process (60).

One immunohistochemical marker of EMT is Snail1 (61) which is expressed in the fibrotic dermis of individuals affected by FFA (62), indicating that profibrotic cells may be derived at least in part by of EMT, where molecules such as TGFβ could be key. This makes one consider targeting such molecular regulators (or EMT-related miRNA) to arrest or reverse the fibrotic process, whether in skin, liver, lungs or kidneys.

Hormones and trauma: true culprits or mere helpers and enemies?

FFA is known to almost exclusively affect women of postmenopausal age (1, 2). Of the 150 cases that currently are under our care, the vast majority developed the disorder after menopause, whereas the few younger female patients had iatrogenic, syndromic or biochemical estrogen decline prior to developing FFA (namely ovarectomy, Turner's syndrome or early menopause). Of course, disease chronicity is known to be associated with excessive fibrous tissue deposition, altered tissue architecture, and organ dysfunction and fibrosis *per se* increases in all organs with aging (63, 64). Estrogen has been found to exert antifibrotic effects *in vivo* (65) and act as potent immunomodulator by suppressing central, innate and adaptive immunity in certain cell types (66), although its effects are known to be variable (67). Does menopause simply coincide with the profibrotic processes of aging or does the menopausal estrogen decline cause unfavourable immunomodulation, thereby allowing a lifelong condition to declare itself and come to the fore? The latter may be in keeping with our observation that other body regions in people with FFA are hairless from a much younger age, thus predating the more intense manifestations of the disorder that occur much later. Male FFA may also be consistent with the above hypothesis: serum testosterone levels are known to decrease with ageing, as does its aromatase-induced conversion to estrogen (68, 69). In either scenario, hormone replacement following realisation of disease activity would not possibly be capable to reverse the underlying process of immune privilege collapse and autoantigen driven lymphocytic attack.

The observation that hair transplantation and face-lift surgery re-activates the disease process (70–72) may also be in keeping with the above hypothesis: the disordered immune-mediated collapse of the hair-follicle's privileged integrity may well leave local factors, such as memory T-cells in skin stroma. In line with this, when apparently normal autologous hair follicles are transplanted to a region previously involved with FFA, subjects can develop the

histological hallmarks of the disease (73, 74). Any traumatic or inflammatory insult or merely implanting more antigens by transplanting hairs in that stroma would be germane to stepping into an active but quiet minefield before the ensuing catastrophe becomes macroscopically and clinically noticeable.

Are there potential diagnostic markers or disease biomarkers for FFA?

MicroRNAs (miRNAs) are relatively recently discovered small, endogenous, non-coding ribonucleic acid molecules (RNAs) that affect various cellular processes underlying health and disease by exerting target gene expression regulatory functions (75, 76). Recent years have seen miRNAs emerging as key determinants of organ phenotypes and potential candidates of prognostic, diagnostic and therapeutic interest in numerous disease processes (77–81). Aberrant expression or function of miRNAs has been implicated in a broad spectrum of human disorders, such as several types of malignancy, neurodegenerative, infectious, metabolic, chronic inflammatory, fibrotic and autoimmune diseases (82–89) and this has led to widespread attention to miRNAomics.

It has recently been discovered that miRNAs can be detected in plasma and serum, where they circulate in a stable form (90–92) mostly because of their encapsulation in serum microvesicles or exosomes (93). There is relative paucity of microRNAomic investigations in cutaneous pathophysiology with the notable exception of psoriasis (94) rendering the prospect of exploring their potential usefulness in FFA and other related alopecic disorders a very attractive one and we are pursuing.

Proposed strategies for advancing insights into FFA

In order to dissect the molecular etiopathogenesis of FFA, a genomic investigation would need to explore if there is a genetic component underlying any observed heritability (**Table 1**). Whether rare coding alleles of large effect size determine FFA would best be addressed via deep sequencing affected familial cases. By studying related, highly clinically consistent individuals it is anticipated that the shared phenotype is caused by shared genetic variation, an approach that circumnavigates the problem of heterogeneity. Nevertheless, the vast majority of cases are sporadic and it would be important to explore whether common genetic variation or more complex pathways predispose to disease: a genome-wide

association study may be the best research tool in delineating underlying pathways, while also examining gene-environment interactions, an approach we are currently recruiting for. The overall rare incidence argues against an environmental etiology as the sole pathogenic determinant, although carefully designed observational studies could examine this notion, ideally followed by epigenetic exploration.

Identifying a refined genetic or protein micro-entity as a biomarker would be indispensable to disease prognostication and could help optimise therapeutic strategies: circulating microRNA and metabolomic profiling may also be an appropriate approach but may be challenging to validate, given the slowly progressive nature of FFA requiring a prolonged and carefully controlled study.

Appreciation of the normal scalp cutaneous cell demographics and architecture and regional gene expression differences could potentially help understand the pattern of disease: appropriate transcriptomic analysis and suitable functional organ and *in vivo* models would be essential and complementary to genomic investigation. *Sine qua non* to molecular delineation is the necessity for deep phenotyping and understanding the clinical character of FFA. To this end, is it time for Cicatricial Alopecia Registry UK (CAR-UK) or, why not, CAR-EU? We have set the foundation stone by establishing a UK-wide research network via the NIHR UK Rare Disease Research Consortium Agreement and look forward to gaining new momentum in the important but thus far understudied area of rare alopecic, scarring and lichenoid dermatoses.

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CT, DAF, CMS, MAS and JAM take full responsibility of the contents of the manuscript. CT and JAM wrote the paper. CMS contributed to Fig 1 and Fig 2. CT, DAF, CMS, MAS and JAM provided critical revision of the manuscript.

Author contributions

Jimenez, Poblet and Izeta take full responsibility of the contents of the manuscript. Jimenez and Izeta wrote the paper. Poblet provided Fig. 1 and Izeta provided Fig. 2. Critical revision of the manuscript for important intellectual content: Jimenez, Poblet and Izeta. Administrative support: Jimenez.

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Conflicts of interest

None declared

REFERENCES

1. Kossard S. Postmenopausal frontal fibrosing alopecia. Scarring alopecia in a pattern distribution. *Arch Dermatol* 1994; **130**:770-4
2. Kossard S, Lee MS, Wilkinson B. Postmenopausal frontal fibrosing alopecia: a variant of lichen planopilaris. *J Am Acad Dermatol* 1997; **36**: 59-66
3. Tan KT. Messenger AG. Frontal fibrosing alopecia: clinical presentation and prognosis. *Br J Dermatol* 2009; **160**: 75-9
4. Moreno-Ramirez D, Camacho Martinez F. Frontal fibrosing alopecia: a survey in 16 patients. *J Eur Acad Dermatol Venereol* 2005; **19**: 700-5
5. Samrao A, Chew AL, Price V. Frontal fibrosing alopecia: a clinical review of 36 patients. *Br J Dermatol* 2010; **163**: 1296-300
6. Camacho Martinez F, Garcia-Hernandez MJ, Mazuecos Blanca J. Post-menopausal frontal fibrosing alopecia. *Br J Dermatol* 1999; **140**: 1181-2
7. Chew AL, Bashir SJ, Wain EM, *et al*. Expanding the spectrum of frontal fibrosing alopecia: a unifying concept. *J Am Acad Dermatol* 2010; **63**: 653-60
8. Vañó-Galván S, Molina-Ruiz AM, Serrano-Falcón C, *et al*. Frontal fibrosing alopecia: a multicenter review of 355 patients. *J Am Acad Dermatol* 2014; **70**: 670-8
9. Eudy G, Solomon AR. The histopathology of noncicatricial alopecia. *Semin. Cutan. Med Surg* 2006; **25**: 35–40.
10. Sperling LC. Alopecia areata. In *An atlas of hair pathology with clinical correlations*. New York, NY: The Parthenon Publishing Group, 2003; 63–78.
11. Poblet E, Jimenez F, Pascual A, Pique E. Frontal fibrosing alopecia versus lichen planopilaris: a clinicopathological study. *Int J Dermatol* 2006; **45**: 375-80
12. MacDonald A, Clark C, Holmes S. Frontal fibrosing alopecia: a review of 60 cases. *J Am Acad Dermatol* 2012; **67**: 955-61

13. Griffin LL, Michaelides C, Griffiths CE, *et al*. Primary cicatricial alopecias: a U.K. survey. *Br J Dermatol* 2012; **167**: 694-7
14. Pandhi D, Singal A, Bhattacharya SN. Lichen planus in childhood: a series of 316 patients. *Pediatr Dermatol* 2014; **31**: 59-67
15. Holmes S, MacDonald A. Frontal fibrosing alopecia*. J Am Acad Dermatol 2014;* **71**: 593-4
16. Dlova N, Goh CL, Tosti A. Familial frontal fibrosing alopecia. *Br J Dermatol 2013*; **168**: 220-2
17. Junqueira Ribeiro Pereira AF, Vincenzi C, Tosti A. Frontal fibrosing alopecia in two sisters. *Br J Dermatol* 2010; **162**: 1154-5
18. Navarro-Belmonte MR, Navarro-López V, Ramírez-Boscà A, *et al*. Case series of familial frontal fibrosing alopecia and a review of the literature. *J Cosmet Dermatol* 2015; **14**: 64-9
19. Tziotzios C, Fenton DA, Stefanato CM, McGrath JA. Familial frontal fibrosing alopecia. *J Am Acad Dermatol* 2015; **73**: e37
20. Wen Y, Liu Y, Xu Y, *et al*. Loss-of-function mutations of an inhibitory upstream ORF in the human hairless transcript cause Marie Unna hereditary hypotrichosis. *Nat Genet* 2009; **41**: 228-33
21. Petukhova L, Duvic M, Hordinsky M, et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. *Nature* 2010; **466**: 113-8
22. Deapen D, Escalante A, Weinrib L, et *al*. A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 1992; **35**: 311-8
23. http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=13382 (Accessed November 2015)
24. Alarcón-Riquelme ME, Ziegler JT, Molineros J, *et al.* GWAS in an Amerindian ancestry population reveals novel systemic lupus erythematosus risk loci and the role of European admixture. *Arthritis Rheumatol* 2015; Nov **25**
25. Pink AE, Simpson MA, Desai N, *et al*. Mutations in the γ-secretase genes NCSTN, PSENEN, and PSEN1 underlie rare forms of hidradenitis suppurativa (acne inversa). *J Invest Dermatol* 2012; **132**: 2459-61
26. Medawar P. Immunity to homologous grafted skin III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br J Exp Pathol* 1948; **129**: 58-69
27. Paus R, Bertolini M. The role of hair follicle immune privilege collapse in alopecia areata: status and perspective. *J Invest Dermatol* Symp Proc 2013; **16**: S25-7
28. Cotsarelis G, Millar SE. Towards a molecular understanding of hair loss and its treatment. *Trends Mol Med* 2001; **7**: 293-301
29. Christoph T, Muller-Rover S, Audring H, *et al*. The human hair follicle immune system: cellular composition and immune privilege. *Br J Dermatol* 2000; **142**: 862-873
30. Ito T, Ito N, Bettermann A, *et al*. Collapse and restoration of MHS class-I-dependent immune privilege: exploiting the human hair follicle as a model.

Am J Pathol 2004; *164*: 623-634
31. Ito T, Ito N, Saatoff M, *et al*. Maintenance of hair follicle privilege is linked to prevention of NK cell attack. *J Invest Dermatol* 2008; *128*: 1196-1206
32. Paus R, Nickoloff BJ, Ito T. A 'hairy' privilege. *Trends Immunol* 2005; **26**: 32-40
33. Breitkopf T, Lo BK, Leung G, et al. Somatostatin expression in human hair follicles and its potential role in immune privilege. *J Invest Dermatol* 2013; **133**: 1722-1730
34. Gilhar A, Etzioni A, Paus R. Alopecia areata. *N Engl J Med* 2012; **366**: 1515-1525
35. Kang H, Wu WY, Lo BK, *et al*. Hair follicles from alopecia areata patients exhibit alterations in immune privilege-associated gene expression in advance of hair loss. *J Invest Dermatol* 2010; **130**: 2677-2680
36. Harries MJ, Sun J, Paus R, *et al*. Management of alopecia areata. *Br Med J* 2010; **341**: c3671
37. Meyer KC, Klatte JE, Dinh HV, *et al*. Evidence that the bulge region is a site of relative immune privilege in human hair follicles. *Br J Dermatol* 2008; **159**: 1077-1085
38. Harries MJ, Paus R. The pathogenesis of primary cicatricial alopecias. *Am J Pathol* 2010; **177**: 2152-2162
39. Ohyama M. Primary cicatricial alopecia: recent advances in understanding and management. *J Dermatol* 2012; **39**: 18-26
40. Harries MJ, Meyer K, Chaudhry I, *et al*. Lichen planopilaris is characterised by immune privilege collapse of the hair follicle's epithelial stem cell niche. *J Pathol* 2013; **231**: 236-47
41. Karnik P, Tekeste Z, McCormick TS, *et al*. Hair follicle stem cell-specific PPARγ deletion causes scarring alopecia. *J Invest Dermatol* 2009; **129**: 1243-1257
42. Varga J, Abraham D. Systemic sclerosis: a prototypic multi-system fibrotic disorder. *J Clin Invest* 2007; **117**: 557-567
43. Denton CP, Black CM. Scleroderma: clinical and pathological advances. *Best Pract Res Clin Rheumatol* 2004; **18**: 271-290
44. Gleeson MH, Taylor S, Dowling RH. Multifocal fibrosclerosis. *Proc Roy Soc Med* 1970; **63**: 1309-1311
45. Neild GH, Rodriguez-Justo M, Wall C, Connolly JO. Hyper-IgG4 disease: report and characterization of a new disease. *BMC Med* 2006; **4**: 23
46. White J.M., Creamer D., du Vivier A.W., Pagliuca A., Ho A.Y., Devereux S., Salisbury J.R., Mufti G.J. Scleroderma graft-versus-host disease: clinical spectrum and therapeutic challenges. Br J Dermatol.2007;156:1032–1038
47. Cowper SE. Nephrogenic systemic fibrosis: an overview. *J AM Coll Radiol* 2008; **5**: 23-28
48. Mendoza FA, Artlett CM, Sandorfi N, *et al*. Description of 12 cases of nephrogenic fibrosing dermopathy and review of the literature. *Semin Arthritis Rheum* 2006; **35**: 238-249
49. Noble PW. Idiopathic pulmonary fibrosis: natural history and prognosis. *Clin Chest Med* 2006; **27**: S11–S16
50. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218
51. Schnaper HW, Kopp JB. Renal fibrosis. *Front Biosci* 2003; **8**: e68-e86
52. Rosenbloom J, Castro SV, Jimenez SA. Narrative review: fibrotic diseases:

cellular and molecular mechanisms and novel therapies. *Ann Intern Med* 2010; **152**: 159-166
53. Wei J, Bhattacharyya S, Tourtellotte WG, Varga J. Fibrosis in systemic sclerosis: emerging concepts and implications for targeted therapy. *Autoimmun Rev* 2011; **10**: 267-275
54. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; **214**: 199-210
55. Krieg T, Abraham ., Lafyatis R. Fibrosis in connective tissue disease: the role of the myofibroblast and fibroblast-epithelial cell interactions. *Arthritis Res Ther* 2007; **9**: S4
56. Neilson EG. Mechanisms of disease: fibroblasts: a new look at an old problem. *Nat Clin Pract Nephrol*2006; **2**: 101-108
57. Abraham DJ, Eckes B, Rajkumar V, Krieg T. New developments in fibroblast and myofibroblast biology: implications for fibrosis and scleroderma. *Curr Rheumatol Rep* 2007; **9**: 136-143
58. McAnulty RJ. Fibroblasts and myofibroblasts: their source, function and role in disease. *Int J Biochem Cell Biol* 2007; **39**: 666-671
59. Piera-Velazquez S, Li Z, and Jimenez SA. Role of Endothelial-Mesenchymal Transition (EndoMT) in the Pathogenesis of Fibrotic Disorders. *Am J Pathol* 2011; **179**: 1074-1080.
60. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; **139**:871-90
61. Nakamura M, Tokura Y Expression of Snail1 in the fibrotic dermis of postmenopausal frontal fibrosing alopecia: possible involvement of an epithelial-mesenchymal transition and a review of the Japanese patients. *Br J Dermatol* 2010; **162**: 1152-4
62. Nakanuma Y, Sato Y, Kiktao A. Pathology and pathogenesis of portal venopathy in idiopathic portal hypertension: hints from systemic sclerosis. *Hepatol Res* 2009; **39**: 1023-1031
63. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Patho*l 2008; 214: 199-210
64. Kapetanaki MG, Mora AL, Rojas M. Influence of age on wound healing and fibrosis. *J Pathol* 2013; **229**: 310-322
65. Yasuda M, Shimizu I, Shiba M, Ito S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology* 1999; **29**: 719-727
66. Nadkarni S, McArthur S. Oestrogen and immunomodulation: new mechanisms that impact on peripheral and central immunity. *Curr Opin Pharmacol* 2013; **13**: 576-81
67. Walker SE**.** Estrogen and autoimmune disease. *Clin Rev Allergy Immunol* 2011; **40**: 60-5
68. Pirke KM, Sintermann R, Vogt HJ. Testosterone and testosterone precursors in the spermatic vein and in the testicular tissue of old men. Reduced oxygen supply may explain the relative increase of testicular progesterone and 17 alpha-hydroxyprogesterone content and production in old age. *Gerontology* 1980; **26**: 221-30
69. Feldman HA et al. Age trends in the level of serum testosterone and other

hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab* 2002; **87**: 589-98

70. Crisóstomo MR, Crisóstomo MC, Crisóstomo MG, *et al*. Hair loss due to lichen planopilaris after hair transplantation: a report of two cases and a literature review. *An Bras Dermatol* 2011; **86**: 359-62
71. Chiang YZ, Tosti A, Chaudhry IH, *et al*. *Br J Dermatol* 2012; **166**: 666-370
72. Donovan J. Lichen planopilaris after hair transplantation: report of 17 cases. *Dermatol Surg* 2012; **38**: 1998-2000
73. Nusbaum BP, Nusbaum AG. Frontal fibrosing alopecia in a man: results of follicular unit test grafting. *Dermatol Surg* 2010; **36**): 959-962
74. Jiménez F, Poblet E. Is hair transplantation indicated in frontal fibrosing alopecia? The results of test grafting in three patients. *Dermatol Surg* 2013; **39**: 1115-8
75. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 2001; **294**: 858-862.
76. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2006; **94**: 776–80
77. Galasso M, Elena Sana M, Volinia S. Non-coding RNAs: a key to future personalized molecular therapy? *Genome Med* 2010; **2**: 12
78. Williams AH, Liu N, van Rooij E, Olson EN. MicroRNA control of muscle development and disease. *Curr Opin Cell Biol* 2009; **21**: 461-9.
79. Wu X, Piper-Hunter MG, Crawford M, et al. MicroRNAs in the pathogenesis of lung cancer. *J Thorac Oncol* 2009; **4**:1028-34
80. Yang BF, Lu YJ, Wang ZG. MicroRNAs and apoptosis: implications in the molecular therapy of human disease. *Clin Exp Pharmacol Physiol* 2009; **36**: 951-60.
81. Zorio E, Medina P, Rueda J, *et al*. Insights into the role of microRNAs in cardiac diseases: from biological signalling to therapeutic targets. *Cardiovasc Hematol Agents Med Chem* 2009; **7**: 82-90
82. Zhang B, Farwell MA. microRNAs: a new emerging class of players for disease diagnostics and gene therapy. *J Cell Mol Med* 2008; **12**: 3-21
83. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Dev Biol* 2007; **302**: 1–12
84. Krutzfeldt J, Stoffel M. MicroRNAs: a new class of regulatory genes affecting metabolism. *Cell Metab* 2006; **4**: 9-12
85. Eacker SM, Dawson TM, Dawson VL. Understanding microRNAs in neurodegeneration. *Nat Rev Neurosci* 2009; **10**: 837-841
86. Pauley KM, Cha S, Chan EK. MicroRNA in autoimmunity and autoimmune diseases. *J*

Autoimmun 2009; **32**: 189-194

87. Chung AC, Lan HY. MicroRNAs in renal fibrosis. *Front Physiol* 2015; **6**: 50
88. Patel V, Noureddine L. MicroRNAs and fibrosis. *Curr Opin Nephrol Hypertens* 2012; **21**: 410-6
89. Yang G, Yang L, Wang W, *et al*. Discovery and validation of extracellular/circulating microRNAs during idiopathic pulmonary fibrosis disease progression. *Gene* 2015; **562**: 138-44
90. Gilad S, Meiri E, Yogev Y, *et al*. Serum microRNAs are promising novel biomarkers *PLoS One* 2008; 3: e3148
91. Chen X, Ba Y, Ma L, *et al*. Characterization of microRNAs in serum: A novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006
92. Mitchell PS, Parkin RK, Kroh EM, *et al*. Circulating microRNAs as stable blood-based markers for cancer detection *Proc Natl Acad Sci USA* 2008; **105**:10513–10518
93. Hunter MP, Ismail N, Zhang X, *et al*. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* 2008; e**3694**: 3
94. Hawkes JE, Nguyen GH, Fujita M, *et al*. microRNAs in Psoriasis. *J Invest Dermatol* 2016; **136:** 365-71

Table 1. A summary of research questions and potential strategies for working and alternate hypothesis

Hypothesis	**Proposed Experimental Strategy**
1. Familial FFA is determined by rare, large effect size, coding alleles	Whole-exome sequencing
2. Sporadic FFA is due to common genetic variation	Genome-wide association study (GWAS)
3. Sporadic FFA is due to environmental factors	Observational/cross-sectional study; Epigenome-wide association study (EWAS)
4. FFA is a complex trait attributed to interplay between genetic and environmental contribution	Gene-environment interaction across the genome via GWAS and multiple testing correction for each interaction
5. The pattern of FFA may be explained by regional differences conferring immune privilege advantage for follicular units in certain areas of the scalp	Characterization of normal skin for cutaneous cell populations immunohistochemically and for gene and protein expression via RNA sequencing and proteomic analysis

FIGURE LEGENDS

Figure 1: Frontal fibrosing alopecia: Scalp with frontal hairline recession involving the temporal areas bilaterally (A), as well as the eyebrows (B). Histopathology shows perifollicular fibrosis with a moderately dense perifollicular lymphoid cell infiltrate involving several hair follicles X40, (C). Detail showing the different phases of the scarring process in different hair follicles leading to hair loss, 100X (D).

Figure 2: Alopecia areata: Retroauricular scalp with a patch of non-scarring alopecia with exclamation hairs (A), which are best appreciated with trichoscopy (B). Histopathology shows an increased number of telogen hair follicles with peribulbar lymphoid cell infiltrate X40(C). Detail of the peribulbar lymphoid cell infiltrate 'swarm of bees' X400 (D).